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Moderate and Severe Nutrient Restriction Has Divergent Effects on Gonadotroph Function in Orchidectomized Sheep[1]

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ABSTRACT

The effect of plane of nutrition and estradiol (E_2) on pituitary concentrations of GnRH receptor and GnRH receptor mRNA was assessed in orchidectomized sheep (wethers). As detailed in the companion paper, 36 wethers were fed to gain, maintain, or lose body weight. Six animals from each feeding group received E_2 (0.31 μg E_2/50 kg per h) or vehicle during Days 51–54 of controlled feeding. Anterior pituitary tissue was collected at the end of infusion. Both moderate and severe nutrient restriction increased ($p < 0.05$) tissue concentrations of FSH and FSHβ mRNA. Conversely, concentrations of GnRH receptor and receptor mRNA were not affected ($p > 0.05$) by plane of nutrition. Estradiol increased ($p < 0.05$) GnRH receptor and receptor mRNA in wethers fed to gain or maintain weight. However, this E_2-induced response was not evident in wethers subject to severe nutrient restriction. These data demonstrate that severe, but not moderate, nutrient restriction suppresses E_2-induced augmentation of tissue concentrations of GnRH receptor and GnRH receptor mRNA. Collectively, the data presented here and in the companion paper suggest that severe nutrient restriction leads to physiologic changes that render the hypothalamus increasingly sensitive to estrogenic stimulation, while the pituitary is made less responsive to steroidal inputs.

INTRODUCTION

The growth and maturation of gonadal tissue and the integrity of reproductive function is closely tied to nutritive status [1–3]. Caloric restriction in adolescent primates [4] and growing animals [5, 6] delays, or prevents, puberty. Similarly, restricted food intake in mature primates [7–9] and other postpubertal animals [2, 10, 11] can lead to cessation or attenuation of reproductive function. In women, for example, starvation or prolonged restriction of caloric intake results in amenorrhea [7]. In males, similar nutritive deficiency results in marked reduction in serum concentrations of LH and testosterone [8, 9, 11] and may lead to reduced spermatogenic activity.

The factors that effect communication between the nutritive and reproductive systems have not been precisely defined [1, 12, 13]. However, it seems likely that starvation or chronic undernourishment alters the complement of metabolic signals that bathe the tissues regulating reproductive processes [12, 13]. This diet-induced change in metabolic status may then affect the functional integrity of the hypothalamo-pituitary-gonadal axis. Indeed, food deprivation, or pharmacological inactivation of fatty acid oxidation and/or glucose utilization, interrupts cyclic reproductive function and inhibits the activity of GnRH-containing neurons in the hypothalamus [10, 14, 15].

In addition to actions expressed at hypothalamic loci, the effects of nutritional deficiency may also be manifest at hypophyseal sites. We have recently demonstrated that estradiol (E_2) increases tissue concentrations of GnRH receptor and GnRH receptor mRNA in sheep fed ad libitum [16]. An objective of the present study was to examine the effect of nutritive status on the magnitude of this E_2-induced response. An additional objective was to assess the effect of food restriction on gonadotropin synthesis. We hypothesized that nutrient restriction would decrease the concentration of gonadotropins and gonadotropin subunit mRNA in pituitary tissue. We also hypothesized that the magnitude of the estrogen-induced increase in steady-state concentrations of GnRH receptor and GnRH receptor mRNA would be reduced in sheep subject to chronic moderate or severe nutrient restriction.

MATERIALS AND METHODS

Experimental Design

The experimental design is presented in detail in the companion paper [17]. Briefly, 36 orchidectomized lambs (wethers; initial weight = 42.3 ± 0.6 kg) were randomly assigned to groups (n = 12 wethers/feeding group) fed to gain, maintain, or lose body weight (groups G, M, and L, respectively) during a 7-wk period of controlled feeding. All lambs were placed in individual pens, and a pelleted diet composed of cereal grains (40%), alfalfa (59%), and trace mineral salt (1%) was fed twice daily. Animals in group G had unlimited access to the pelleted diet. In contrast, the amount of diet provided to wethers in groups M and L was gradually reduced during the period of controlled feeding. Wethers in group M were fed to maintain initial body weight, while animals in group L were fed to lose weight at a constant rate during the 7-wk feeding period.

Six lambs from each feeding group received E_2 (0.31 μg E_2/50 kg per h in 10% ethanol-saline [vehicle]) as a continuous 72-h infusion during Days 51–54 of controlled feeding. As noted in the companion paper [17] and elsewhere [18], this rate of E_2 delivery establishes a serum concentration of E_2 of approximately 2–3 pg/ml. Controls from each feeding group (n = 6) received a comparable volume of vehicle alone.

At the conclusion of the 72-h infusion period, animals were stunned by a captive bolt pistol and killed by exsanguination at the University of California (UC) Davis Slaughter Facility. Anterior pituitary tissue was quickly excised and halved by a midsagittal cut, and each half was immediately frozen in liquid nitrogen and stored at −80°C for later analysis. All experimental procedures involving the use of animals were conducted in accordance with NIH Guidelines and were reviewed and approved by the Animal Use and Care Committee for the UC.

Endocrine Analysis

The affinity and concentration of GnRH receptor in pituitary tissue were determined by means of the procedure

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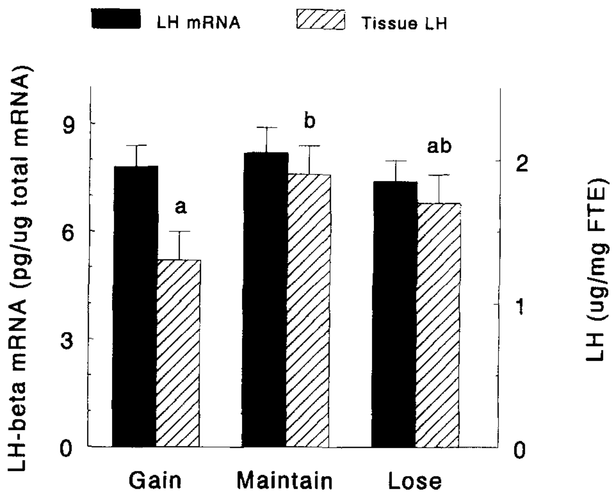

FIG. 1. Concentrations of LH and LHβ mRNA in pituitary tissue of wethers at the conclusion of a 7-wk period of controlled feeding. During controlled feeding, wethers were fed to gain, maintain, or lose weight (n = 12 wethers/feeding group). E_2 did not alter tissue concentrations of LH and LHβ mRNA. Therefore, data from wethers receiving E_2 or vehicle are combined within feeding groups. Tissue concentrations of LH that do not share a letter designation differed significantly ($p < 0.05$).

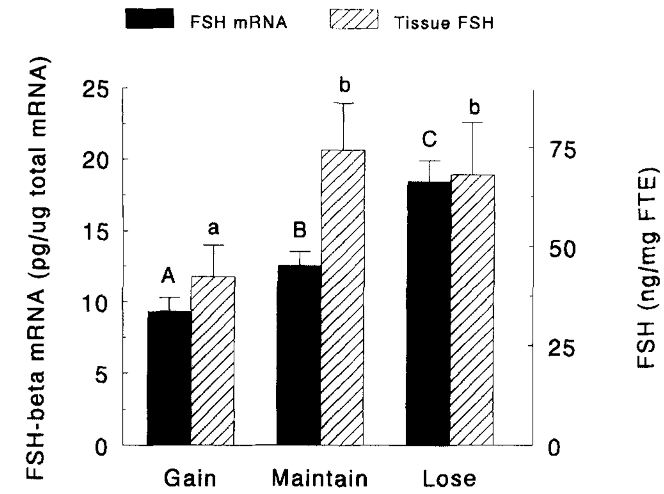

FIG. 2. Concentrations of FSH and FSHβ mRNA in pituitary tissue of wethers at the conclusion of a 7-wk period of controlled feeding. During controlled feeding, wethers were fed to gain, maintain, or lose weight (n = 12 wethers/feeding group). E_2 did not alter tissue concentrations of FSH and FSHβ mRNA. Therefore, data from wethers receiving E_2 or vehicle are combined within feeding groups. Tissue concentrations of FSH and FSHβ mRNA that do not share a letter designation differed significantly ($p < 0.05$).

described previously [19]. The concentration of LH and FSH in homogenates of pituitary tissue was determined using validated RIA procedures [20, 21]. The LH (NIAMDD-oLH-25) and FSH (NIAMDD-oFSH-RP-1) reference standards were gifts from the National Hormone and Pituitary Program (Baltimore, MD). In all cases, intra- and interassay coefficients of variation were less than 10%. The minimum sensitivity of the LH and FSH assays was 0.1 and 0.25 ng/ml, respectively.

Tissue concentrations of mRNA for the gonadotropin subunits and GnRH receptor mRNA were determined by use of the solution hybridization procedures described previously [16, 22]. Briefly, total RNA was isolated by homogenization of frozen blocks of hemipituitary tissue (150–300 mg) in 5 ml 4.0 M guanidinium thiocyanate. The homogenate was extracted with acidified phenol, and total RNA was precipitated with ethanol. Isolated RNA was dissolved in sterile water treated with diethyl pyrocarbonate (DEPC) and stored at −80°C for later analysis. Extraction yield was 3.5–4.5 mg RNA/g tissue.

Plasmids containing cDNA inserts for the bovine LH [23] and FSHβ [24] subunits were kindly provided by Dr. R. Maurer (Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR). A plasmid containing a cDNA insert for the ovine GnRH receptor [25] was kindly provided by Dr. J. Brooks (MRC Reproductive Biology Unit, Edinburgh, UK). The sense and antisense cRNAs were generated by in vitro transcription using either T7 RNA or SP6 RNA polymerase and the Riboprobe Gemini System II reagent system (Promega Corp., Madison, WI).

Statistical Analysis

Wethers were assigned to one of 6 cells in a 3 × 2 factorial experiment, with the factors of interest being diet and E_2 exposure (n = 6 wethers per cell). The data were analyzed by ANOVA [26]. Where significant treatment effects were noted, mean comparisons were made using Duncan's multiple range test. Data are presented in the text as mean ± SEM. Pituitary tissue concentrations of the gonadotropins and mRNA encoding the LHβ and FSHβ subunits were not significantly affected by exposure to E_2; therefore, these data were pooled within feeding group.

RESULTS

Concentration of Gonadotropins and Gonadotropin Subunit mRNA

The concentration of LH mRNA in pituitary tissue of ad libitum fed wethers did not differ ($p > 0.05$) from the concentration noted in wethers after 7 wk of moderate or severe nutrient restriction (Fig. 1). In contrast, the concentration of LH in pituitary tissue of wethers subject to moderate food restriction was increased ($p < 0.05$) relative to the level noted in ad libitum fed animals. The final pituitary concentration of LH in wethers subject to severe nutrient restriction was intermediate between the values noted for ad libitum fed and moderately restricted wethers.

In contrast to LHβ mRNA, tissue concentrations of FSHβ mRNA, when expressed relative to values in ad libitum fed wethers, were increased ($p < 0.05$) by 7 wk of moderate nutrient restriction (Fig. 2). More severe nutrient restriction further accentuated this change in tissue concentration of FSHβ mRNA. Similarly, the concentration of FSH in pituitary tissue of wethers subject to moderate or severe food restriction was significantly increased, relative to the value noted in ad libitum fed animals.

Tissue Concentration of GnRH Receptor

The concentration of GnRH receptor in pituitary tissue was not affected ($p > 0.05$) by plane of nutrition in wethers receiving vehicle alone during the 72-h infusion period (Fig. 3). In contrast, continuous delivery of E_2 increased ($p < 0.05$) GnRH receptor concentration in full-fed wethers and wethers subject to moderate nutrient restriction. Conversely, E_2 infusion for 72 h did not significantly increase the tissue concentration of GnRH receptor in wethers subject to severe nutrient restriction during the period of controlled feeding. In contrast to tissue concentration of GnRH

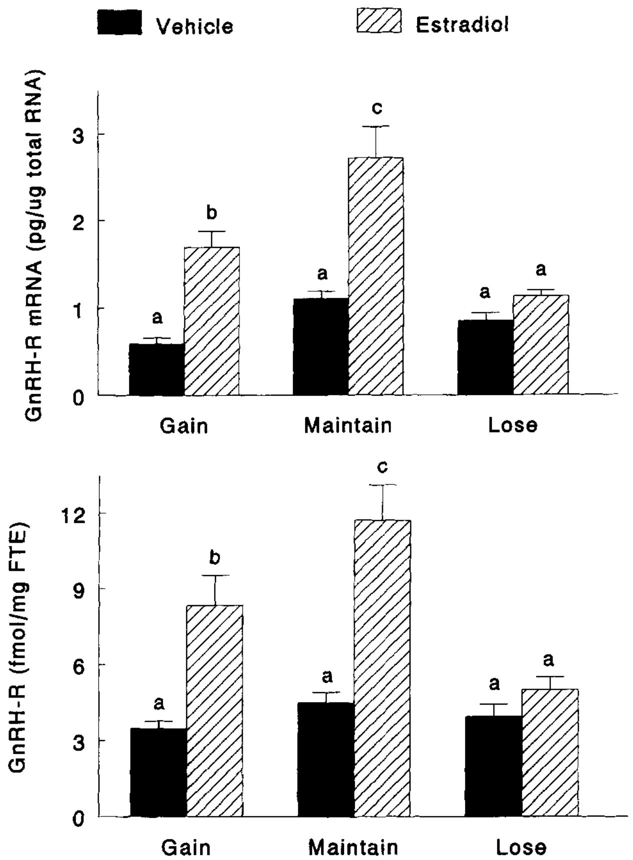

FIG. 3. Effect of continuous infusion of E_2 (0.31 μg/50 kg/h) or vehicle (10% ethanol-saline) on steady-state concentrations of GnRH receptor and GnRH receptor mRNA in pituitary tissue of orchidectomized sheep (wethers) fed to gain, maintain, or lose weight during a 7-wk period of controlled feeding ($n = 6$ wethers/group). Anterior pituitary tissue was collected at the end of a 72-h infusion that was initiated on Day 51 of controlled feeding. Tissue concentrations of GnRH receptor and GnRH receptor mRNA that do not share a letter designation differed significantly ($p < 0.05$).

receptor, receptor affinity ($K_a = 1.8 \pm 0.1 \times 10^9$ L/M) was not significantly affected by plane of nutrition or E_2.

Steady-State Concentration of GnRH Receptor mRNA

The concentration of GnRH receptor mRNA in pituitary tissue did not differ ($p > 0.05$) with nutritional status in wethers receiving vehicle during the infusion period (Fig. 3). As with GnRH receptor, tissue concentrations of GnRH receptor mRNA were significantly increased by E_2 infusion in full-fed wethers. Moderate nutrient restriction potentiated this E_2-induced change in GnRH receptor mRNA. In contrast, the tissue concentration of GnRH receptor mRNA was not significantly affected by E_2 administration in wethers subject to severe nutrient restriction during the period of controlled feeding.

DISCUSSION

We examined gonadotroph function in full-fed wethers and wethers subject to moderate or severe nutrient restriction during a 7-wk period of controlled feeding. Although we predicted that chronic nutrient restriction would decrease tissue stores of the gonadotropins and the concentration of gonadotropin subunit mRNA, these measures of gonadotroph function were either unaffected or increased in wethers subject to moderate or severe food restriction. These observations are generally consistent with previous work using rodents, postpartum cattle, and ovariectomized (OVX) sheep that demonstrated that chronic nutrient restriction did not significantly alter tissue stores of the gonadotropins or pituitary concentrations of gonadotropin subunit mRNA [27–30]. Conversely, the concentration of FSH, but not LH, was markedly decreased in pituitary tissue of growth-restricted OVX lambs [31]. Moreover, tissue concentrations of mRNA encoding the gonadotropin subunits were significantly lower in this animal model than in lambs after 14 days of realimentation [31]. As noted in the companion paper [17], this qualitative difference in gonadotroph response to chronic nutrient restriction is probably due to difference in severity of nutritive stress and/or variation between research models in age and body composition at initiation of restricted feeding.

It is interesting to note that serum concentrations of LH and LH pulse frequency are also markedly reduced in growth-retarded lambs [5, 32]. This suggests that the activity of the GnRH pulse-generating system is reduced in this model. Indeed, Prasad and coworkers [33] reported that the amplitude of secretory bursts of GnRH was significantly attenuated in growth-retarded lambs. This reduction in GnRH stimulation induced by nutritive stress is likely to account for much of the reduction in tissue concentrations of LH and FSH and gonadotropin subunit mRNA noted in this animal model. Consistent with this postulate are reports that episodic delivery of GnRH augments tissue stores of the gonadotropins and increases gonadotropin subunit mRNA in pituitary tissue of OVX sheep [34] and male [35, 36] and female [37] rats during acute or chronic food restriction.

As noted in the companion paper [17] and elsewhere [28, 30, 38, 39], episodic secretion of LH and, presumably, pulsatile secretion of GnRH persist in mature sheep and rats during prolonged nutrient restriction. This persistent pulsatile release of GnRH, albeit at altered amplitude or frequency, is likely to account for the maintenance of tissue concentrations of gonadotropins and gonadotropin subunit mRNA during chronic nutrient restriction. It is interesting to note that tissue concentrations of FSH and FSHβ mRNA were markedly increased in wethers subject to prolonged moderate or severe nutrient restriction. This diet-induced augmentation of pituitary stores of FSH may contribute to the increased serum concentrations of FSH noted in wethers subject to moderate or severe nutrient restriction [17].

Similar augmentation of tissue concentrations of FSH and FSHβ mRNA is also reported during glucocorticoid stimulation [40, 41]. Other researchers have noted that chronic nutrient restriction is often associated with modest, but significant, increases in serum concentrations of glucocorticoids [9, 42–45]. We postulate that the increase in FSH gene expression noted in these studies is a consequence of persistent stimulation of adrenal function during nutritive stress. Alternatively, the augmentation of pituitary concentrations of FSH and FSHβ mRNA during nutrient restriction may be a consequence of a nutrition-dependent change in the activity of the GnRH pulse generator. As noted in the companion paper [17], severe nutrient restriction reduced the frequency and increased the amplitude of secretory episodes of LH. A similar change in the pattern of GnRH stimulation selectively increased the concentration of FSHβ mRNA in the pituitary tissue of rats [46, 47].

In contrast to FSH and FSHβ mRNA, tissue concentrations of GnRH receptor and GnRH receptor mRNA are not affected by chronic nutrient restriction. Indeed, we note here that, in the absence of estrogenic inputs, the concen-

trations of GnRH receptor and GnRH receptor mRNA in pituitary tissue of wethers did not vary with nutritive status. Tissue concentrations of GnRH receptor were also unaffected by plane of nutrition in OVX sheep [48] and postpartum cattle [29]. Maintenance of tissue concentrations of GnRH receptor during restricted feeding is likely to account, at least in part, for the ability of undernourished animals to respond to exogenous GnRH stimulation [6, 42].

We have noted in previous studies that the concentrations of GnRH receptor and GnRH receptor mRNA in pituitary tissue of full-fed wethers are sensitive to estrogenic stimulation [16, 18]. Interestingly, this estrogen-induced response is evident, and even amplified, during periods of moderate nutrient restriction. However, the stimulatory effect of E_2 on tissue concentrations of GnRH receptor and GnRH receptor mRNA is not evident during severe nutritive stress. This estrogen-induced increase in tissue concentrations of GnRH receptor and GnRH receptor mRNA is likely to reflect direct actions of the gonadal steroid at hypophyseal loci since the E_2-induced response is manifest in sheep in which hypothalamic inputs have been eliminated by hypothalamo-pituitary disconnection [49, 50], immunoneutralization [19], or administration of a GnRH antagonist [51]. In addition, an E_2-induced increase in GnRH receptor and GnRH receptor mRNA is noted in ovine pituitary cells in culture [52–54]. Collectively, these observations indicate that E_2 acts directly at hypophyseal loci to increase steady-state concentrations of GnRH receptor and GnRH receptor mRNA in gonadotroph cells.

This stimulatory response to E_2 at hypophyseal loci runs counter to the negative feedback effect, expressed at hypothalamic sites, that leads to a decrease in activity of the GnRH pulse generator. These divergent estrogen-induced responses appear to differ in steroid sensitivity since the stimulatory effect of E_2 on tissue concentrations of GnRH receptor and GnRH receptor mRNA is expressed without a concurrent change in the episodic nature of LH secretion [16, 18]. Indeed, we report here that E_2-induced augmentation of GnRH receptor and GnRH receptor mRNA is evident in full-fed wethers and wethers subject to moderate nutrient restriction without appreciable change in the pattern of LH and, by inference, GnRH secretion. From these observations, we conclude that E_2-induced up-regulation of GnRH receptor and GnRH receptor mRNA is normally more sensitive to estrogenic inputs than is the opposing hypothalamic response, negative feedback on the GnRH pulse-generating system. However, an apparent shift in the sensitivity of these estrogenic responses was noted in wethers subject to severe nutrient restriction. In this group, estrogenic action at hypothalamic loci was evident (decrease in frequency of secretory episodes of LH), but the stimulatory response to E_2 at hypophyseal loci was not.

This suggests that chronic and severe nutrient restriction leads to physiologic changes in hypothalamic and pituitary function that render the hypothalamus increasingly sensitive to estrogenic stimulation, while the pituitary is made less responsive to steroidal inputs. The physiologic basis for this functional transformation can not be determined from the data presented here; however, our working hypothesis suggests that chronic nutrient restriction alters the concentration and/or character of the E_2 receptors present in target cells in the hypothalamus and pituitary. The identification of multiple isoforms of the estrogen receptor in hypothalamic and pituitary tissue of sheep and rats [55–57] is consistent with this hypothesis. In addition, the distribution of these E_2 receptor variants is tissue-specific and varies with gonadal status. We extend these observations by suggesting that nutritive status also alters the distribution of E_2 receptor isoforms in the hypothalamus and pituitary, leading to depressed estrogenic action at pituitary loci and enhanced sensitivity to the negative feedback effects of E_2 at hypothalamic loci during severe nutrient restriction. Supporting this hypothesis is the recent observation that food deprivation or pharmacological restriction of nutrient availability selectively alters the number of neurons expressing the estrogen receptor in several hypothalamic nuclei [58].

Taken together, the observations presented here and in the companion paper [17] indicate that chronic nutrient restriction has profound effects on hypothalamic and pituitary loci. Moderate or severe nutrient restriction enhances the secretory activity of gonadotroph cells and leads to a marked increase in tissue concentrations of FSH and FSHβ mRNA. In addition, the negative feedback potency of E_2 is increased and the stimulatory action of E_2 at hypophyseal sites is suppressed during severe, but not moderate, nutrient restriction. The mechanisms underlying the divergent effects of moderate and severe nutrient restriction on hypothalamic and pituitary function are topics requiring additional study.

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